Supplemental Information

A Relationships between enzyme masses and flux control coefficients

According to Brown [12], in the state of minimum total enzyme concentrations, the flux control coefficient of any enzyme will be proportional to that enzyme concentration for linear metabolic pathways. This relationship is given in Equation (S1) where C_i^J represents a scaled flux control coefficient.

$$\frac{C_{E_i}^J}{E_i} = c. \tag{S1}$$

To give more insight in this relationship, the derivation of this equation will be explained with the two-enzyme metabolic network shown in Figure S1.

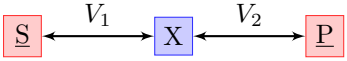


Figure S1: Simplest biochemical pathway with two enzymes (E_1 and E_2), two fixed metabolites (S and P), and one floating metabolite (X). Floating and fixed species are indicated in blue and red squares, respectively. Fixed species are underlined.

Reducing E_1 by an infinitesimal amount (δE_1) results in a pathway flux decrease. An increase of E_2 by an infinitesimal amount (δE_2) is necessary to return the flux to its original rate. For this model, we assume the model is in the state of minimum total enzyme concentrations for a particular flux. In other words, the minimum total enzyme concentration is found for a particular flux, which means that Equation (S2) must hold.

$$\delta E_1 + \delta E_2 = 0. \tag{S2}$$

Otherwise, enzyme concentrations can be adjusted such that the total enzyme concentrations decreases and the flux remains constant. Because $\delta J = 0$, the relationship in Equation (S3) must be true.

$$\frac{\delta J}{J} = \frac{C_1^J}{E_1}\delta E_1 + \frac{C_2^J}{E_2}\delta E_2 = 0. \tag{S3}$$

Rearranging Equation (S3), in observation of Equation (S2) gives Equation (S4). This Equation can be generalized into the earlier given Equation (S1).

$$\frac{C_1^J}{E_1} = \frac{C_2^J}{E_2}. \tag{S4}$$

However, Klipp and Heinrich [13] show that this relationship is not valid for branched pathways, and derived a new relationship between flux control coefficients and enzyme concentrations in the state of minimum total enzyme concentration. This relationship is given in Equation (S5). Here, $\vec{C}$ and $\vec{e}$ represent the flux control coefficients matrix and the enzyme concentration vector, respectively. See [13] for a proof of this relationship.

$$\vec{C}^T\vec{e} = \vec{e}. \tag{S5}$$

From this equation, Equation (S1) can be derived as follows:

$$\begin{pmatrix} C_1^{J_1} & C_2^{J_1} \\ C_1^{J_2} & C_2^{J_2} \end{pmatrix}^T \begin{pmatrix} E_1 \\ E_2 \end{pmatrix} = \begin{pmatrix} E_1 \\ E_2 \end{pmatrix} \tag{S6}$$

$$\begin{aligned} C_1^{J_1}E_1 + C_1^{J_2}E_2 &= E_1 \\ C_2^{J_2}E_1 + C_2^{J_2}E_2 &= E_2. \end{aligned} \tag{S7}$$

Since $J_1 = J_2$ in the linear pathways, the above equations can be simplified:

$$\begin{aligned} C_1^J(E_1 + E_2) &= E_1 \\ C_2^J(E_1 + E_2) &= E_2 \end{aligned} \tag{S8}$$

$$\begin{aligned} C_1^J &= \tfrac{E_1}{E_T} \\ C_2^J &= \tfrac{E_2}{E_T}, \end{aligned} \tag{S9}$$

where

$$E_T = \sum E_i. \tag{S10}$$

Metabolic cost of enzymes can, however, vary due to their different molar masses. As a consequence, weight factors need to be introduced into Equation (S10). This relationship is given in Equation (S11), where m_i is the molar mass ($g \times mole^{-1}$) of E_i, and M_T the total enzyme mass.

$$M_T = \sum E_i m_i = \sum M_i. \tag{S11}$$

The addition of the molar masses to Equation (S10) results in the modification of Equations (S1) and (S5). We illustrate this with the linear model shown in Figure S1. Reducing E_1 by an infinitesimal amount (δE_1) results in an increase of E_2 by an infinitesimal amount (δE_2). In an optimized state — minimum total enzyme mass for a particular flux — Equation (S12) must hold:

$$\delta M_T = \delta E_1 m_1 + \delta E_2 m_2 = 0. \tag{S12}$$

Moreover, $\delta J = 0$ in the optimized case, which is given by Equation (S3). Rearranging Equation (S3), in observation of Equation (S12) gives Equation (S13).

$$\frac{C_1^J}{E_1 m_1} = \frac{C_2^J}{E_2 m_2}. \tag{S13}$$

Next, Equation (S13) can be generalized to Equation (S14). This relationship shows that, in the state of minimum total enzyme concentrations for a given flux, the flux control coefficient of any enzyme will be proportional to that enzyme mass.

$$\frac{C_i^{J_k}}{E_i m_i} = c. \tag{S14}$$

This relationship is similar to Equation (S1), whereas it incorporates the effect of molar mass. A similar adjustment was done for Equation (S5):

$$\vec{C}^T \vec{e}\, \vec{m} = \vec{e}\, \vec{m}, \tag{S15}$$

where the i-th element of $\vec{e}\, \vec{m}$ is $E_i m_i$.

From Equation (S15), one can derive Equation (S14) for linear pathways as follows:

$$\begin{pmatrix} C_1^{J_1} & C_2^{J_1} \\ C_1^{J_2} & C_2^{J_2} \end{pmatrix}^T \begin{pmatrix} E_1 \\ E_2 \end{pmatrix} \begin{pmatrix} m_1 \\ m_2 \end{pmatrix} = \begin{pmatrix} E_1 \\ E_2 \end{pmatrix} \begin{pmatrix} m_1 \\ m_2 \end{pmatrix} \tag{S16}$$

$$\begin{aligned} C_1^{J_1} E_1 m_1 + C_1^{J_2} E_2 m_2 &= E_1 m_1 \\ C_2^{J_2} E_1 m_1 + C_2^{J_2} E_2 m_2 &= E_2 m_2. \end{aligned} \tag{S17}$$

Since $J_1 = J_2$ in linear pathways,

$$\begin{aligned} C_1^J(E_1 m_1 + E_2 m_2) &= E_1 m_1 \\ C_2^J(E_1 m_1 + E_2 m_2) &= E_2 m_2 \end{aligned} \tag{S18}$$

$$\begin{aligned} C_1^J &= \tfrac{E_1 m_1}{M_T} \\ C_2^J &= \tfrac{E_2 m_2}{M_T} \end{aligned} \tag{S19}$$

$$\tfrac{C_1^J}{E_1 m_1} = \tfrac{C_2^J}{E_2 m_2} = \tfrac{1}{M_T}. \tag{S20}$$

B Maximize metabolic flux given a total enzyme mass constraint

In this section, we propose an optimization algorithm, which was used to optimize steady-state flux given a finite total enzyme mass. In such an optimized state, according to Equation (S14), flux control coefficients will be proportional to enzyme mass. We developed an algorithm that finds, independent from initial conditions, the highest flux through a metabolic pathway. This algorithm iteratively increases enzyme concentrations with low $C_i^{J_k} \times (E_i m_i)^{-1}$ and decreases enzyme concentrations with high $C_i^{J_k} \times (E_i m_i)^{-1}$. Here, k represents the flux to optimize. Eventually, $C_i^{J_k} \times (E_i m_i)^{-1}$ converges for each enzyme in the branch to optimize, such that Equation (S14) holds when this algorithm is finished redistributing enzyme concentrations.

Note that enzyme concentrations can tend to zero if the network has, for instance, more than one independent flux. Because this algorithm makes percentage changes to enzyme concentrations, enzyme concentrations can go asymptotically to zero. For this reason, only $C_i^{J_k} \times (E_i m_i)^{-1}$ are taken into account if the enzyme concentration is greater than a threshold close to zero. Enzymes favorable in optimized states can be close to zero in non-optimized states. Therefore, an exception is made for enzymes that start with an enzyme concentration less than the selected threshold, which they lose if they start to decrease.

The first step of this algorithm is to perform steady-state analysis. Secondly, scaled flux control coefficients are determined and divided by the current enzyme concentrations times the corresponding molar mass. Subsequently, the algorithm proposes to increase the enzyme with the highest $C_i^{J_k} \times (E_i m_i)^{-1}$ and decrease the enzyme with the lowest $C_i^{J_k} \times (E_i m_i)^{-1}$. Proposed changes in enzyme concentration are based on the enzyme with the lowest $E_i m_i$. Moreover, these proposed changes must be sufficiently small, because flux control coefficients alter when enzyme concentrations are altered. Because of the constraint given in Equation (S11), Equation (S21) must hold while redistributing enzyme concentrations.

$$\delta M_T = m_{max}\delta E_{max} + m_{min}\delta E_{min} = 0 \tag{S21}$$

Here, E_{max} and E_{min} represent the enzymes with the maximum and minimum $C_i^{J_k} \times (E_i m_i)^{-1}$ where E_i is greater than the threshold. Hence, the proposed change for the enzyme with the lowest $C_i^{J_k} \times (E_i m_i)^{-1}$ is given by Equation (S22).

$$\delta E_{max} = -E_{min}\frac{m_{min}}{m_{max}} \tag{S22}$$

During every iteration the concentration of two enzymes changes, while the total enzyme mass remains constant. The system, subsequently, goes to a new steady-state with a corresponding flux. The optimal enzyme distribution is found once the flux to optimize starts to decrease. An example of this numerical algorithm is shown in Figure S2.

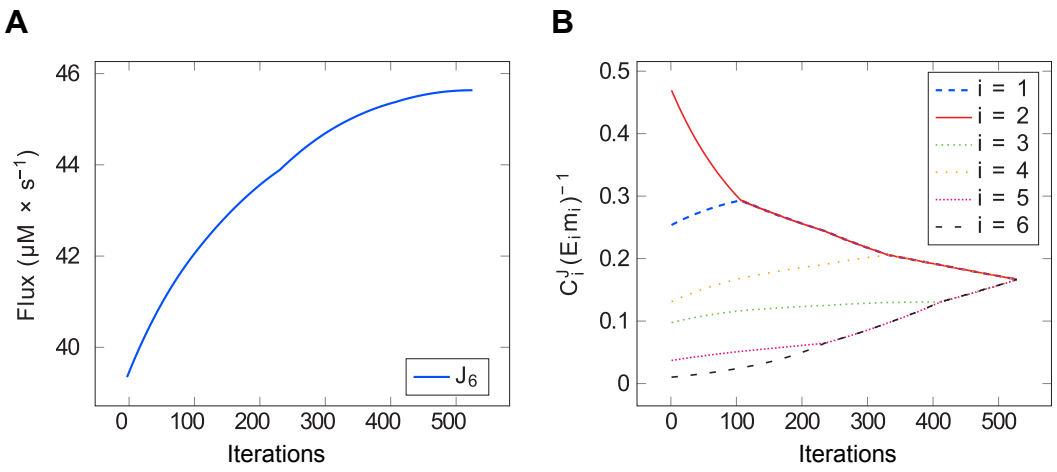


Figure S2: Simulation results for the maximization of pathway flux for a six-enzyme linear pathway. (a) Increasing pathway flux, (b) Converging $C_i^{J_k} \times (E_i m_i)^{-1}$.

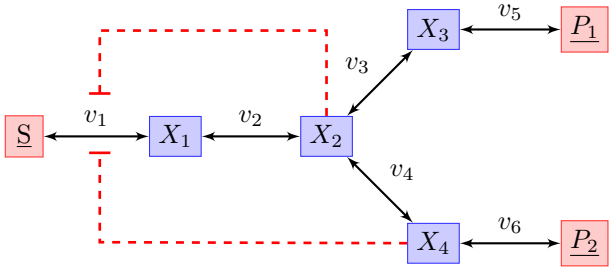


Figure S3: Branched metabolic network. Case 1: no feed-back, case 2: feed-back from X_2 to V_1, and case 3: feed-back from X_4 to V_1.

C New relationships between flux control coefficients and enzyme concentrations for branched networks

This section describes numerical results supporting new relationships between flux control coefficients and enzyme concentrations similar to the Brown's result, Equation (S1). Our result presented here is fundamentally different from the Klipp's result in that it provides extra information than Equation (S5) can do.

First, consider case I of the branch network described in Figure S3. We minimized total enzyme mass under the constraint of fixed fluxes. Figure S4 illustrates the system's behavior. Figure S4(a) confirms that the fluxes remained constant during the simulation. Figure S4(b) shows that some enzyme levels increased, while others decreased during the minimization of total enzyme mass. Figures S4(c)–(f) illustrate $C_i^{J_k}(E_i m_i)^{-1}$ values during the optimization procedure. As can be seen from these figures, $C_i^{J_k}(E_i m_i)^{-1}$ values converged for linear segments in branched pathways, just as they do in linear pathways as was shown previously by Brown [12]. These results suggest that Equation (S14) also holds for linear segments in branched pathways.

$$\begin{aligned}
\frac{C_3^{J_3}}{E_3} = \frac{C_5^{J_3}}{E_5} = \frac{C_3^{J_5}}{E_3} = \frac{C_5^{J_5}}{E_5} = c_1 \\
\frac{C_4^{J_4}}{E_4} = \frac{C_6^{J_4}}{E_6} = \frac{C_4^{J_6}}{E_4} = \frac{C_6^{J_6}}{E_6} = c_2 \\
\frac{C_1^{J_1}}{E_1} = \frac{C_2^{J_1}}{E_2} = \frac{C_1^{J_2}}{E_1} = \frac{C_2^{J_2}}{E_2} = c_3.
\end{aligned} \tag{S23}$$

We performed simulations (not presented here) for larger branched networks up to twenty metabolites, where the model contained more than two branches. Results, again, indicated that Equation (S14) holds for linear segments in branched pathways in the state of minimal enzyme mass for a particular set of fluxes. This suggests that Equation (S14) can be modified into Equation (S24).

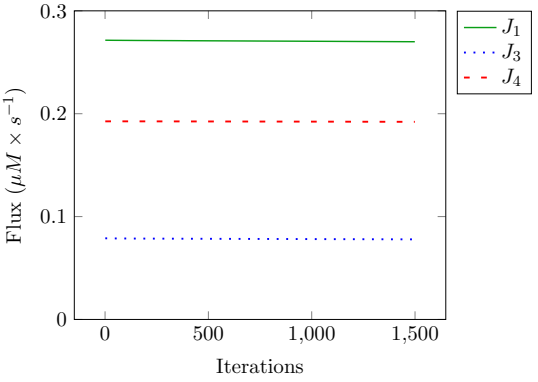
$$\frac{C_i^{J_k}}{E_i m_i} = c, \tag{S24}$$

if k and i are in the same branch.

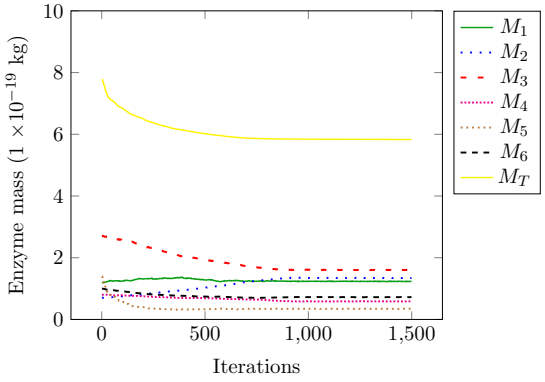
We further investigated the network shown in Figure S3, but now with different types of feed-back (e.g. case II and III). These results are illustrated in Figures S5 and S6. Here, $C_i^{J_k}(E_i m_i)^{-1}$ did not convergence for branches where the negative feed-back originated, which suggests that Equation (S24) need to be adjusted into Equation (S25).

$$\frac{C_i^{J_k}}{E_i m_i} = c, \tag{S25}$$

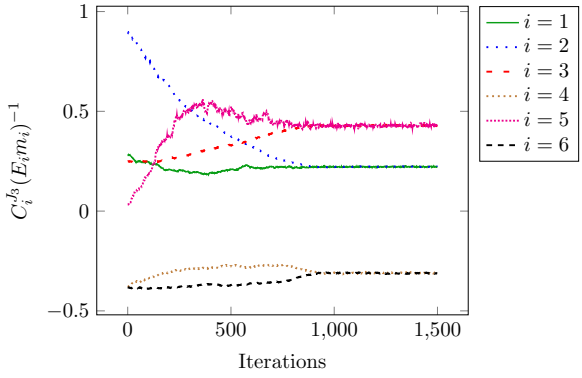
if k and i are in the same branch without an originating feed-back.



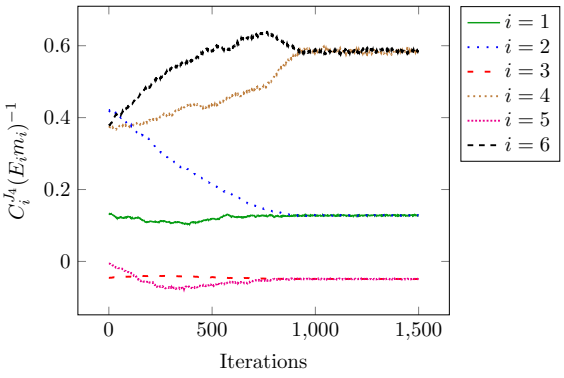
(a) Fluxes remained constant



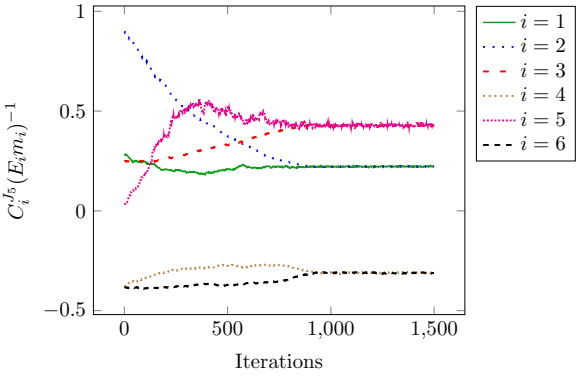
(b) Enzyme mass (1×10^{-18} kg) altering due to minimization of total enzyme concentration.



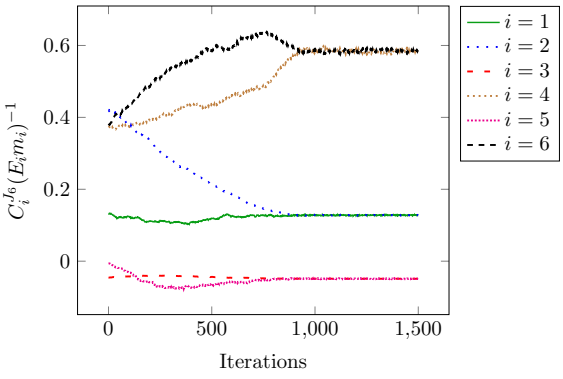
(c) Convergence of $C_i^{J_3}(E_i m_i)^{-1}$



(d) Convergence of $C_i^{J_4}(E_i m_i)^{-1}$

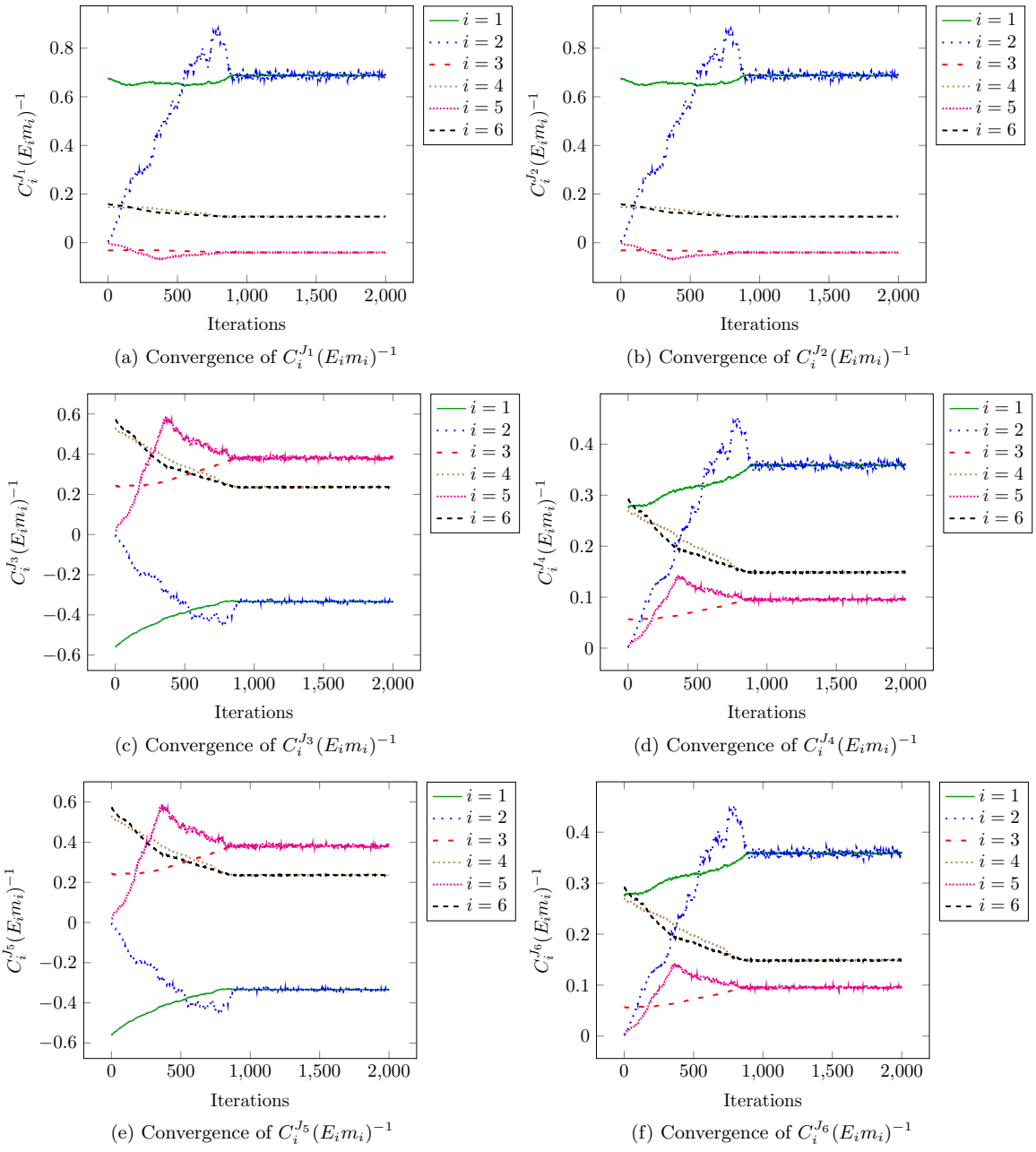


(e) Convergence of $C_i^{J_5}(E_i m_i)^{-1}$



(f) Convergence of $C_i^{J_6}(E_i m_i)^{-1}$

Figure S4: Simulation results for the minimization of total enzyme concentration while fixing fluxes for a six-enzyme branched pathway without any feed-back (Figure S3. The $C_i^{J_k}(E_i m_i)^{-1}$ of reaction sets from different branches — v_1 and v_2, v_3 and v_5, and v_4 and v_6 — did converge. For parameters, see Section F.4.2.



(a) Convergence of $C_i^{J_1}(E_i m_i)^{-1}$

(b) Convergence of $C_i^{J_2}(E_i m_i)^{-1}$

(c) Convergence of $C_i^{J_3}(E_i m_i)^{-1}$

(d) Convergence of $C_i^{J_4}(E_i m_i)^{-1}$

(e) Convergence of $C_i^{J_5}(E_i m_i)^{-1}$

(f) Convergence of $C_i^{J_6}(E_i m_i)^{-1}$

Figure S5: Simulation results for the minimization of total enzyme concentration while fixing fluxes for a six-enzyme branched pathway with negative feed-back from X_2 to v_1 (Figure S3. The $C_i^{J_k}(E_i m_i)^{-1}$ of reaction sets from different branches — v_1 and v_2, v_3 and v_5, and v_4 and v_6 — did converge. For parameters, see Section F.4.2.

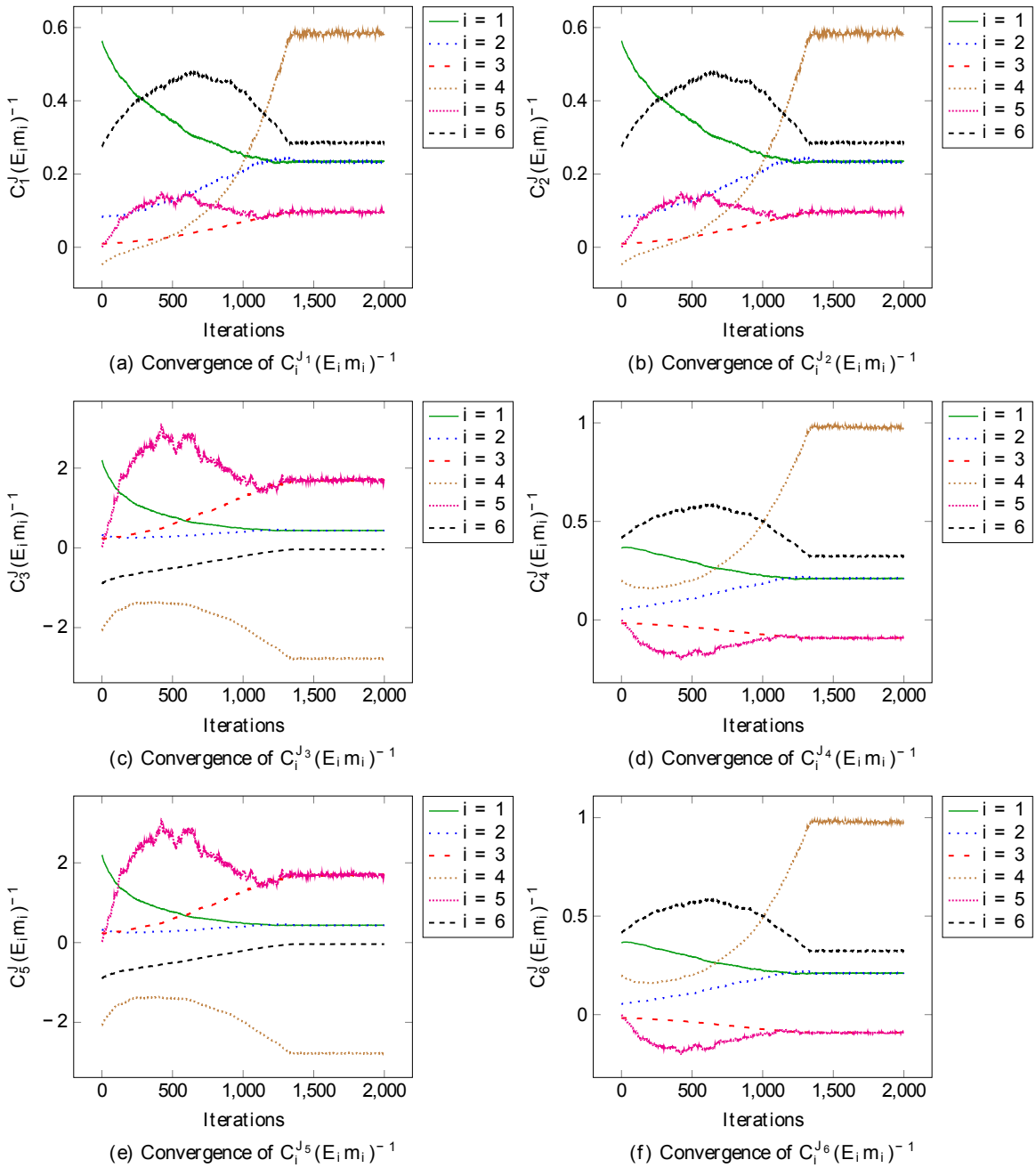


Figure S6: Simulation results for the minimization of total enzyme concentration algorithm for a six-enzyme branched pathway with negative feed-back from X_4 to v_1 (Figure S3). The $C_i^{J_k}(E_i m_i)^{-1}$ of reaction set v_4 and v_6 did not converge. For parameters, see Section F.4.2.

D Distribution of the deviation from the desired input-output characteristics

The fitness of each GRN was based on the deviation from the desired input-output characteristics. Specifically, the deviation was quantified by the relative difference between the desired and training output values. The distribution of these relative deviation values within the trained region was significantly different from a normal distribution (Shapiro-Wilk test [**?**] showed $p \ll 1.2 \times 10^{-5}$). Quantile-Quantile plots given in Figure S7 show that the distributions are rather similar to a gamma distribution, thus the standard deviations were determined based on a gamma distribution.

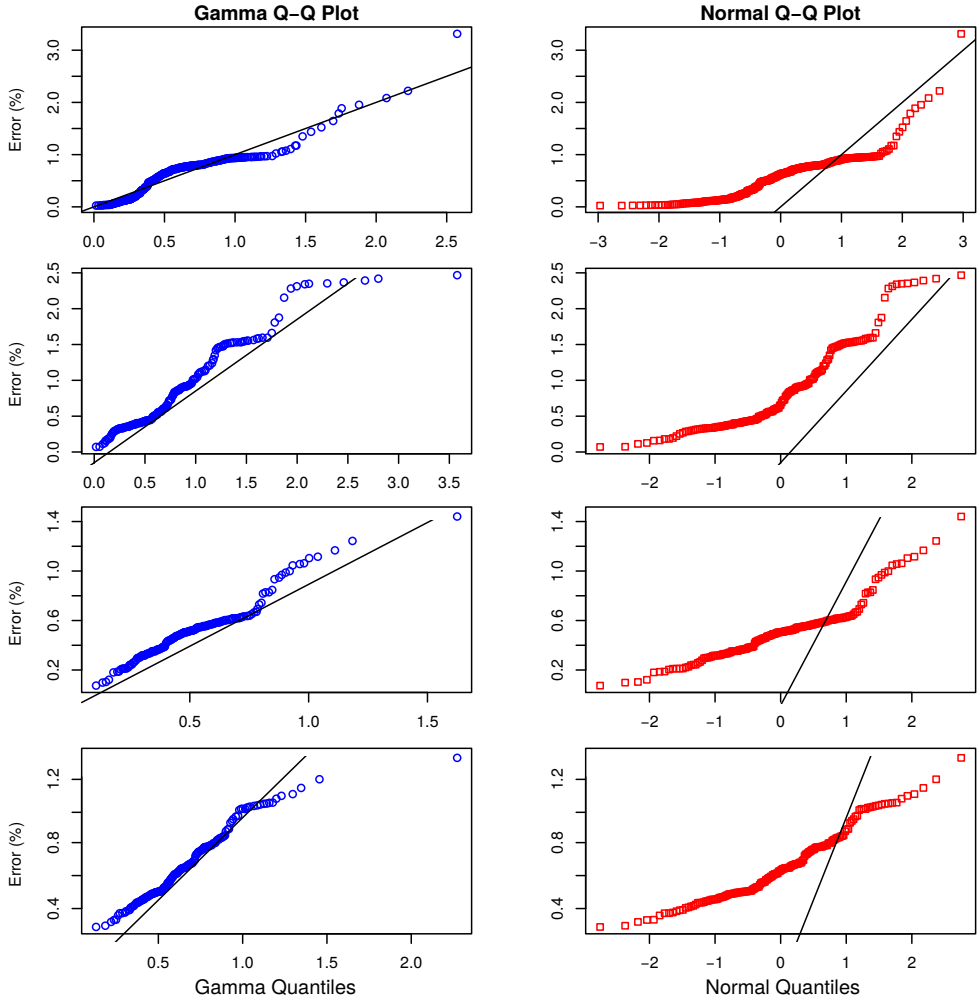


Figure S7: Quantile-Quantile (Q-Q) plots of the relative deviation values within the trained region against gamma and normal distributions. Shape parameter k equals 2.25, 2.25, 5.75, and 7.0 respectively.

E Perturbing concentrations of boundary metabolites

In this section, we illustrate how large the differences between optimal enzyme concentrations can be for different concentrations of boundary metabolites. During training of GRNs the concentrations of boundary metabolite S and P were often between of $1 - 100\ \mu M$ and $0.01 - 1\ \mu M$, respectively. In Figure S8 we show the optimal enzyme concentration given a total enzyme mass constraint for two particular enzymes. Figure S8(a) shows that perturbation of S has a large effect on the optimal enzyme concentration, while perturbing P has almost no effect. In contrast, Figure S8(b) shows that both S and P have a significant effect on the optimal enzyme concentration of this particular enzyme.

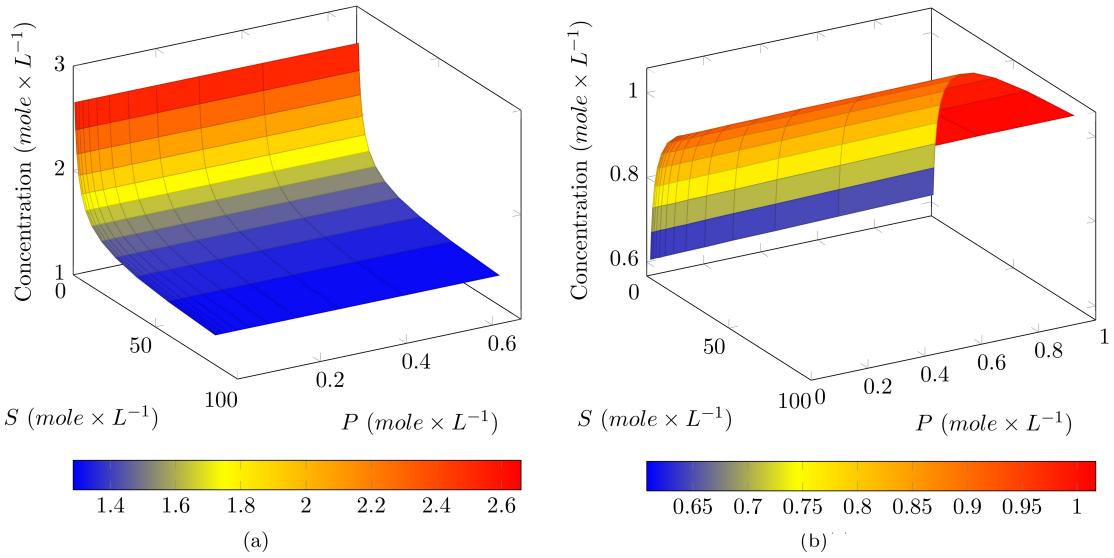


Figure S8: Relationships between concentrations of boundary metabolites S and P and two particular enzymes.(a) Perturbing S has a much larger effect than P on the optimal enzyme concentration of this enzyme. (b) Perturbing both S and P has a large effect on the optimal enzyme concentration of this enzyme.

F Models of metabolic and gene regulatory networks

F.1 Two-enzyme metabolic network

```
# PySCeS input file
# Easy Pathway

FIX: S P

R1:
    S  = X
    E1*kcat1*(S - X/Keq1)/(S + KmS*(1 + X/KmX))
R2:
    X = P
    E2*kcat2*(X - P/Keq2)/(X + KmX*(1 + P/KmP))


# InitExt
S  = 75
P  = 25

# InitPar
E1  = 1
E2  = 1
m1  = 1
m2  = 1

kcat1 = 1000
kcat2 = 500

Keq1 = 5
Keq2 = 22.5

KmS = 1
KmX = 3
KmP = 2

# InitVar
X  = 1
```

F.2 Three-enzyme metabolic network

```
FIX: S P

R1:
    S = X
    E1*kcat1*(S - X/Keq1)/(S + KmS*(1 + X/KmX)+ alpha*Y/KmY)

R2:
    X  = Y
    E2*kcat2*(X - Y/Keq2)/(X + KmX*(1 + Y/KmY))

R3:
    Y = P
    E3*kcat3*(Y - P/Keq3)/(Y + KmY*(1 + P/KmP))

# InitExt
S  = 10
P  = 0.01

# InitPar
E1  = 1
E2  = 1
E3  = 1
m1 = 1
m2 = 1
m3 = 1

kcat1 = 500
kcat2 = 50
kcat3 = 100
alpha = 3

KmS = 1
KmX = 0.5
KmY = 1.5
KmP = 1
Keq1 = 20
Keq2 = 15
Keq3 = 50

# InitVar
X  = 1
Y  = 1
```

F.3 Five-enzyme metabolic network

```
FIX: S P

R1:
    S  = X1
    E1*kcat1*(S - X1/Keq1)/(S + KmS*(1 + X1/KmX))
R2:
    X1 = X2
    E2*kcat2*(X1- X2/Keq2)/(X1 + KmX*(1 + X2/KmX))
R3:
    X2 = X3
    E3*kcat3*(X2 - X3/Keq3)/(X2 + KmX*(1 + X3/KmX))
R4:
    X3 = X4
    E4*kcat4*(X3 - X4/Keq4)/(X3 + KmX*(1 + X4/KmX))
R5:
    X4  = P
    E5*kcat5*(X4 - P/Keq5)/(X4 + KmX*(1 + P/KmP))

# Fixed Species
S  = 1
P  = 1

# Variable Species
X1 = 1
X2 = 1
X3 = 1
X4 = 1

E1 = 0.7
E2 = 1.3
E3 = 1
E4 = 1
E5 = 1

# Parameters
kcat1 = 100
kcat2 = 50
kcat3 = 100
kcat4 = 75
kcat5 = 125
KmS = 5
KmX = 0.5
KmP = 10
Keq1 = 20
Keq2 = 15
Keq3 = 10
Keq4 = 20
Keq5 = 15
```

F.4 Six-enzyme metabolic networks

F.4.1 Linear with feed-back

```
FIX: S P

R1:
    S  = X1
    E1*kcat1*(S - X1/Keq1)/(S + KmS*(1 + X1/KmX1))
R2:
    X1 = X2
    E2*kcat2*(X1- X2/Keq2)/(X1 + KmX1*(1 + X2/KmX2))
R3:
    X2 = X3
    ((E3*kcat3*X2/KmX2)*(1-X3/(X2*Keq3))*(X2/KmX2 + X3/KmX3)**(n-1))/
    (((X2/KmX2+ X3/KmX3)**n) + (1+(X5/KmX5)**n)/(1+alpha*(X5/KmX5)**n))
R4:
    X3 = X4
    E4*kcat4*(X3 - X4/Keq4)/(X3 + KmX3*(1 + X4/KmX4))
R5:
    X4 = X5
    E5*kcat5*(X4 - X5/Keq5)/(X4 + KmX4*(1 + X5/KmX5))
R6:
    X5  = P
    E6*kcat6*(X5 - P/Keq6)/(X5 + KmX5*(1 + P/KmP))

# Fixed Species
S  = 100
P  = 0.5

# Variable Species
E1 = 1
E2 = 1
E3 = 1
E4 = 1
E5 = 1

E6 = 1
X1 = 1
X2 = 1
X3 = 1
X4 = 1
X5 = 1

# Parameters
kcat1 = 70
kcat2 = 50
kcat3 = 100
kcat4 = 75
kcat5 = 125
kcat6 = 150
KmS = 5
KmX1 = 0.5
```

```
KmX2 = 1.5
KmX3 = 4
KmX4 = 2
KmX5 = 0.75
KmP = 7
Keq1 = 20
Keq2 = 15
Keq3 = 10
Keq4 = 20
Keq5 = 15
Keq6 = 30
n = 1.5
alpha = 1
```

F.4.2 Branched

```
FIX: S P1 P2

R1:
    S  = x1
    ((E1*k1f*S/Ks)*(1-x1/(S*Keq1))*(S/Ks + x1/Kx1))/
    (((S/Ks + x1/Kx1)) + (alpha1*(x4/Kx5))+(alpha2*(x3/Kx5)))
R2:
    x1 = x2
    E2*k2f*(x1 - x2/Keq2)/(x1 + Kx1*(1 + x2/Kx2))
R3:
    x2 = x3
    E3*k3f*(x2 - x3/Keq3)/(x2 + Kx2*(1 + x3/Kx3))
R4:
    x2 = x4
    E4*k4f*(x2 - x4/Keq4)/(x2 + Kx2*(1 + x4/Kx4))
R5:
    x3 = P1
    E5*k5f*(x3 - P1/Keq5)/(x3 + Kx3*(1 + P1/Kp1))
R6:
    x4 = P2
    E6*k6f*(x4 - P2/Keq6)/(x4 + Kx4*(1 + P2/Kp2))

# InitExt
S  = 10.0
P1 = 1.0
P2 = 1.0

# InitPar
E1 = 1.0
E2 = 1.0
E3 = 1.0
E4 = 1.0
E5 = 1.0
E6 = 1.0
```

```
k1f = 1
k2f = 1.5
k3f = 1.0
k4f = 1.0
k5f = 2.6
k6f = 1.0

Keq1 = 0.7
Keq2 = 1.1
Keq3 = 2.1
Keq4 = 1.4
Keq5 = 0.4
Keq6 = 1.6

m1 = 1.2
m2 = 0.7
m3 = 2.7
m4 = 0.8
m5 = 1.4
m6 = 1.0

Ks  = 10
Kx1 = 5
Kx2 = 2
Kx3 = 0.5
Kx4 = 4
Kp1 = 5
Kp2 = 5
Kx5 = 10

# No feed-back
alpha1 = 0
alpha2 = 0

# Negative feed-back from X4 to v1
# alpha1 = 50
# alpha2 = 0

# InitVar
x1 = 1.0
x2 = 1.0
x3 = 1.0
x4 = 1.0
```

G Gene Regulatory Networks

G.1 Two-enzyme GRN

```
FIX: W1 G1 G2
# Reactions
R1:
    G1 > E1
    Vmax1*(1/(1+k1*E1**h1))*(1/(1+k2*P**h2))*(1/(1+k3*S**h3))
R2:
    G2 > E2
    Vmax2*(1/(1+k4*E2**h4))*(1/(1+k5*E1**h5))
R3:
    E1 > W1
    E1*0.650524
R4:
    E2 > W1
    E2*0.223366

# Fixed Species
W1 = 1
G1 = 1
G2 = 1

# Variable Species
E1 = 1
E2 =1
S = 1
P = 1

# Parameters
Vmax1 = 3.75422
k1 = 6.48859
h1 = 4.30682
k2 = 0.402033
h2 = 1.00019
k3 = 0.43719
h3 = 0.869487
Vmax2 = 2.46437
k4 = 0.86132
h4 = 3.6914
k5 = 4.90228
h5 = 3.14973
```

G.2 Three-enzyme GRN

```
FIX: W1 G1 G2 G3
# Reactions
R1:
    G1 > E1
    Vmax1*((1+k1*P**h1)*(1+k3*E1**h3)-1)/((1+k1*P**h1)*(1+k2*S**h2)*(1+k3*E1**h3))
R2:
    G2 > E2
```

```
    Vmax2*((1+k4*S**h4)-1)/((1+k4*S**h4)*(1+k5*P**h5))
R3:
    G3 > E3
    Vmax3*((1+k6*S**h6)*(1+k7*P**h7)*(1+k8*E3**h8)-1)/
    ((1+k6*S**h6)*(1+k7*P**h7)*(1+k8*E3**h8))
R4:
    E1 > W1
    E1*1.09882
R5:
    E2 > W1
    E2*1.27824
R6:
    E3 > W1
    E3*1.29683

# Fixed Species
W1 = 1
G1 = 1
G2 = 1
G3 = 1

# Variable Species
E1 = 1
E2 = 1
E3 = 1
S = 1
P = 1

# Parameters
Vmax1 = 2.83741
k1 = 1.63564
h1 = 1.49258
k2 = 2.00275
h2 = 0.247268
k3 = 8.92731
h3 = 0.157243

Vmax2 = 3.28334
k4 = 1.01241
h4 = 0.187473
k5 = 0.0641848
h5 = 0.487284

Vmax3 = 1.24576
k6 = 8.5334
h6 = 0.216253
k7 = 9.47921
h7 = 1.63791
k8 = 2.45668
h8 = 3.99067
```

G.3 Five-enzyme GRN

```
FIX: W1 G1 G2 G3 G4 G5
# Reactions
R1:
    G1 > E1
    Vmax1*((1+k2*E1**h2)-1)/((1+k1*E5**h1)*(1+k2*E1**h2))
R2:
    G2 > E2
    Vmax2*((1+k3*P**h3)*(1+k4*S**h4)-1)/((1+k3*P**h3)*(1+k4*S**h4))
R3:
    G3 > E3
    Vmax3*((1+k5*E5**h5)-1)/((1+k5*E5**h5)*(1+k6*E3**h6))
R4:
    G4 > E4
    Vmax4*((1+k7*E5**h7)-1)/((1+k7*E5**h7))
R5:
    G5 > E5
    Vmax5*((1+k8*S**h8)*(1+k9*P**h9)-1)/((1+k8*S**8)*(1+k9*P**h9)*(1+k10*E5**h10))
R6:
    E1 > W1
    E1*0.516899
R7:
    E2 > W1
    E2*0.867095
R8:
    E3 > W1
    E3*1.41811
R9:
    E4 > W1
    E4*0.797611
R10:
    E5 > W1
    E5*0.256305

# Fixed Species
W1 = 1
G1 = 1
G2 = 1
G3 = 1
G4 = 1
G5 = 1

# Variable Species
E1 = 1
E2 = 1
E3 = 1
E4 = 1
E5 = 1
S = 1
P = 1
```

```
# Parameters
Vmax1 = 2.43445
k1 = 6.6671
h1 = 1.54412
k2 = 9.67675
h2 = 2.53249
Vmax2 = 1.24985
k3 = 2.10143
h3 = 0.0121673
k4 = 1.97132
h4 = 1.04927
Vmax3 = 2.30057
k5 = 6.64561
h5 = 1.71241
k6 = 0.433479
h6 = 2.48621
Vmax4 = 1.34903
k7 = 3.16177
h7 = 1.59593
Vmax5 = 0.183229
k8 = 0.896061
h8 = 0.580101
k9 = 0.0406431
h9 = 0.612953
k10 = 0.085952
h10 = 0.938131
```

G.4 Six-enzyme GRN

```
FIX: W1 G1 G2 G3 G4 G5 G6 S P
# Reactions
R1:
    G1 > E1
    Vmax1*((1+k1*E1**h1)-1)/((1+k1*E1**h1)*(1+k2*E6**h2))
R2:
    G2 > E2
    Vmax2*((1+k3*E4**h3)-1)/((1+k3*E4**h3))
R3:
    G1 > E3
    Vmax3*(1/(1+k5*P**h5))*((k6*E6**h6)/(1+k6*E6**h6))
R4:
    G1 > E4
    Vmax4*((k7*E6**h7)/(1+k7*E6**h7))
R5:
    G1 > E5
    Vmax5*((k9*E6**h9)/(1+k9*E6**h9))
R6:
    G1 > E6
    Vmax6*((1+k11*S**h11)*(1+k12*P**h12)-1)/((1+k11*S**h11)*(1+k12*P**h12))
R7:
    E1 > W1
    E1*0.186924
R8:
    E2 > W1
    E2*1.88726
R9:
    E3 > W1
    E3*1.57073
R10:
    E4 > W1
    E4*1.40082
R11:
    E5 > W1
    E5*1.67811
R12:
    E6 > W1
    E6*0.440807

# Fixed Species
S = 100
P = 1
W1 = 1
G1 = 1
G2 = 1
G3 = 1
G4 = 1
G5 = 1
G6 = 1
```

```
# Variable Species
E1 = 1
E2 = 1
E3 = 1
E4 = 1
E5 = 1
E6 = 1

# Parameters
Vmax1 = 1.4411
k1 = 2.40536
h1 = 0.152005
k2 = 8.1128
h2 = 1.40023
Vmax2 = 3.06359
k3 = 7.698
h3 = 1.65138
Vmax3 = 2.01559
k5 = 0.0272647
h5 = 0.106304
k6 = 5.03767
h6 = 0.994795
Vmax4 = 2.07882
k7 = 8.22248
h7 = 1.56628
Vmax5 = 2.15233
k9 = 3.33499
h9 = 1.44571
Vmax6 = 0.244187
k11 = 0.77059
h11 = 0.635955
k12 = 0.028323
h12 = 1.19337
```